AWARD NUMBER: W81XWH-15-1-0361

TITLE: Exaggerated Cap-Dependent Translation as a Mechanism for Corticostriatal Dysfunction in Fragile X Syndrome Model Mice

PRINCIPAL INVESTIGATOR: Claudia Bagni

CONTRACTING ORGANIZATION: VLAAMS INSTITUUT VOOR BIOTECHNOLOGIES
RIJVISSCHESTRAAT 120
GENT 9052

REPORT DATE: October 2016

TYPE OF REPORT: ANNUAL

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2016	Annual	09/30/2015–09/29/2016

4. TITLE AND SUBTITLE

Exaggerated Cap-Dependent Translation as a Mechanism for Corticostriatal Dysfunction in Fragile X Syndrome Model Mice

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-15-1-0361

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Claudia Bagni, Ph.D.
Eric Klann, Ph.D.

En Mail: eklann@cns.nyu.edu, Claudia.Bagni@unil.ch

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

VLAAMS INSTITUUT VOOR BIOTECHNOLOGIES
RIJVISSCHESTRAAT 120
GENT 9052

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Our laboratories are committed to understanding the detailed molecular abnormalities associated with developmental disabilities and how these result in synaptic dysfunction and aberrant behavior. The overall hypothesis is that repetitive and perseverative behaviors exhibited by FXS patients that can be recapitulated in the FXS model mice are caused by affected cortico-striatal synapses. Our specific tasks are centered on a proteomic study of FXS striatal synapses by using a transgenic mouse model that allows to capture "native" post-synaptic densities. So far, we have generated the necessary number of animals, purified the synaptic complexes from different brain areas at two developmental stages and finally set up quantitative western blotting for the NMDA receptor. These experiments now enable us to identify quantitative and qualitative changes in the the protein composition of the post-synaptic densities, which will complement the electrophysiological and behavioral studies performed by the coordinator.

15. SUBJECT TERMS

Nothing listed

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	7	19b. TELEPHONE NUMBER *(include area code)*

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Introduction

Our laboratories are committed to understanding the detailed molecular abnormalities associated with developmental disabilities and how these result in synaptic dysfunction and aberrant behavior. Our overall hypothesis is that repetitive and perseverative behaviors exhibited by FXS patients that can be recapitulated in the FXS model mice are caused by affected cortico-striatal synapses. To test this hypothesis we propose two specific aims: **1) To determine cortico-striatal synaptic composition, function and plasticity in FXS model mice; 2) To determine whether altered cortico-striatal synaptic plasticity and repetitive/perseverative behaviors displayed by FXS model mice are reversed by novel cap-dependent translation inhibitors.** Our specific tasks are centered on a proteomic study of FXS striatal synapses by using a transgenic mouse model that allows to capture "native" synapses. Purified synapse will be analyzed by mass spectrometry and the data will be validated using biochemical and cellular methods. The comparison of the synaptic proteome between the wild type and the FXS mice during development will identify which complexes are affected in FXS and possibly in other synaptopathies. These data will complement the electrophysiological and behavioral studies performed by the coordinator.

Key words

Fragile X syndrome (FXS), synaptic proteome, synaptic structure, postsynaptic density, PSD-95, NMDA receptors.

Accomplishments

Below the accomplishments associated with Task1 in the approved statement of work.

Major goals of the project (year 1)

Major task 1 was to determine the cortico-striatal synaptic composition, function and plasticity, in FXS model mice. These experiments were to be completed in year 1 and 2.
Major task 2 was to determine whether altered cortico-striatal synaptic plasticity and repetitive/perseverative behaviours displayed by FXS model mice are reversed by novel cap-dependent translation inhibitors. This task was to be completed only in year 3. In the following I will report our progress on the specific subtasks of Task1 that were assigned to our unit.

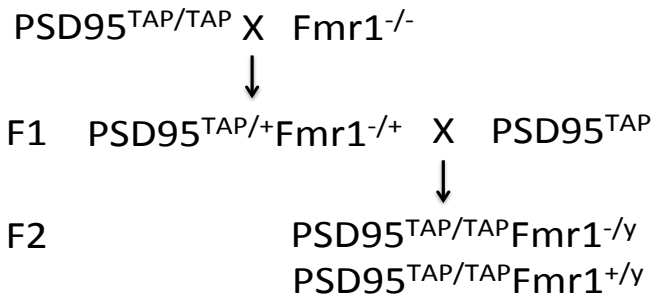

Figure 1. Scheme of the double cross PSD95^{TAP}x Fmr1$^{-/-}$ mice.

Sub task 2- Breed and generate sufficient numbers of Fmr1 KO-PSD95 TAP mice.
In order to generate enough number of mice to isolate the tissue we performed breeding of both colonies PSD95^{TAP} and *Fmr1* KO with the following strategy: Males carrying both alleles of PSD95^{TAP} were bred with females homozygous for *Fmr1* (Fmr1$^{-/-}$) (Figure 1). From the first generation (F1) we selected females PSD95$^{TAP/+}$x Fmr1$^{+/-}$ and crossed them with males homozygous for PSD95$^{TAP/TAP}$. From the second generation (F2) we selected males that were homozygotes for the TAP transgene and Fmr1-/y. PSD95$^{TAP/TAP}$Fmr1$^{+/y}$ animals

were used as controls.

In order to generate the PSD95$^{TAP/TAP}$Fmr1$^{-/y}$ mice and relative controls, during the first year we bred and housed 150 PSD95$^{TAP/TAP}$Fmr1$^{+/y}$ mice and 300 PSD95$^{TAP/TAP}$Fmr1$^{-/y}$ mice. For the MS analyses, male littermates with the correct genotype were sacrificed and brain dissected into three areas: cortex, hippocampus and striatum. Areas were collected, snap-frozen and pooled for the proposed experiments. Currently, more animals are in breeding in order to isolate PSDs for phospho-proteomic and stimulation studies (years 2 and 3 of the grant).

Subtask 3: Measure levels of NMDA-R subunits and complexes at cortico-striatal synapse in FXS model mice and their wild-type littermates.
For this task we set up the NMDA-R detection in quantitative western blotting (data not shown but see Figure 3). The samples that have been set aside from the TAP purification (n = 54, see subtask 4) will be systematically analyzed for NMDAR levels. This will be completed during the second year.

Subtask 4: Isolation of the PSD-95 interactome from the cortical, hippocampal, and cortico-striatal regions of the PSD95$^{TAP/TAP}$Fmr1$^{-/y}$ and controls.
Tissue collection: brain areas were collected from the generated animals to set up the PSD protein complex isolation. While the major goal of this study is to identify and characterize striatal synapses, we have reasoned that the use of the large number of animals should be optimized and therefore we set up parallel studies on hippocampi and cortices. At first, we checked whether the areas hippocampus, cortex and striatum were specifically dissected. We collected the 3 different brain areas at two developmental stages, postnatal day 30 (P30), corresponding to juvenile animals, and at P150, adult animals with mature synapses. We performed protein extraction according to (Fernandez et al., 2009) and we blotted against PSD95, a specific post-synaptic adaptor protein widely expressed in excitatory synapses, tyroxine hydroylase, an enzyme specifically expressed in the striatum, and FMRP to monitor its relative level in the 3 different brain areas. As seen in Figure 2, FMRP is well expressed in all three brain regions, and dopaminergic neurons are mostly found in the striatal fraction.

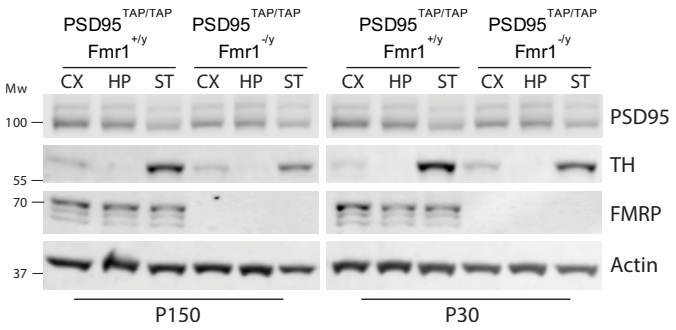

Figure 2. Western blotting to analyze protein extracts from cortex (CX), hippocampus (HP) and striatum (ST) at two developmental stages, postnatal day 150 and 30 (P150, P30). Proteins analyzed: postsynaptic marker PSD95, striatum marker tyrosine hydroxylase (TH), FMRP, and actin as loading control. Mw: molecular weight in kDa; P150: postnatal day 150; P30: postnatal day 30.

Setup of the purifications: Following the tissue collection we set up the conditions to isolate the protein complexes for mass spectrometry (MS) analysis. For all three brain regions, we used conditions previously established for the hippocampal extracts (Fernández et al., 2009). We performed 54 purifications: three brain areas (cortex, striatum and hippocampus), at 2 developmental stages (P30 and P150), from 2 genotypes (PSD-95$^{TAP/TAP}$ x Fmr1$^{+/y}$, 95$^{TAP/TAP}$ x Fmr1$^{-/y}$ mice and WT) and in triplicates.

Protein complexes were isolated with a FLAG antibody, and proteins were eluted following trypsin digestion to be directly injected into the mass spectrophotometer. To monitor the pull-downs, 1/50 of the purified complexes were run into a Western blot and blotted against

the bait, PSD95, and two well-known interactors, NMDA receptor 2B and Arc (Figure 3). Digested peptides were sent to the VIB proteomics expertise center (PEC) in Gent (Belgium) and analyzed by LC-MS/MS. The analysis of the samples is currently undergoing. We expect the first dataset results by the end of 2016. The data will be analyzed with a 3-way Anova to reveal the influence of FMRP deletion, age and brain region.

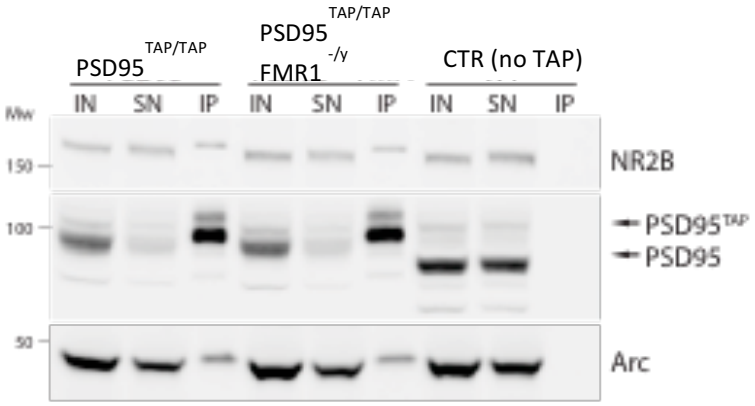

Figure 3. Representative western blot of Tandem Affinity Precipitation of PSD95 complexes from PSD95$^{TAP/TAP}$, PSD95^{TAP}xFmr1$^{-/y}$ and non tagged (CTR) mice. IN: input; SN: supernatant; IP: immunoprecipitation. NMDAreceptor 2B, PSD95 and Arc proteins were detected. Mw: molecular weight in kDa.

Subtasks 7 and 11 are scheduled to be completed in the second year.

Summary of accomplishments

- Generated the necessary number of animals
- Purified the synaptic complexes from different brain areas and at two developmental stages
- Set up quantitative western blotting for NMDAR

Impact

Our laboratories are committed to understanding the detailed molecular abnormalities associated with developmental disabilities and how these result in synaptic dysfunction and aberrant behavior. Thus, military families with members afflicted with these disorders will benefit from these studies. In the short-term, our studies will provide information whether the ***composition and the fine regulation (phosphorylation) of the synapses are different in FXS and non affected mouse models***. In the long-term, our studies will provide information for the design and use of ***novel compounds to therapeutically target*** pathways affected in FXS and other developmental disabilities such as ASD.

Changes/Problems

My laboratory has recently – October 2016 – relocated to the University of Lausanne (Switzerland) where I accepted a position as Chair of the Department of Fundamental Neuroscience. While this change brought about a certain delay, we do not foresee major changes in the time table as listed in the statement of work, and no changes in expenditure except for the hiring of a new scientist to lead this project replacing Esperanza Fernandez. A strong candidate with the suitable profile for this grant is being hired from January 1st, 2017**.** Importantly, the University of Lausanne and my Institute provide an ideal environment to pursue this project at its best**.**

Products

There have been no publications based on this work so far. Preliminary data generated in this work in term of methodology we use and the impact on the understanding of FXS synapses have been presented to a few meetings.

1) Gordon Research Conference "Fragile X and Autism-Related Disorders". Vermont, USA.
2) NCCR Synapsy "The Neurobiology of Mental Health". Geneva, Switzerland
3) ENI-MIT "Synaptic basis of neuron network dysfunction in brain disorders", Göttingen, Germany.
4) 2nd International Gencodys Conference "Integratice Networks in Intellectual Disabilities", Crete, Greece.

Participants and other collaborating organization

Name: Claudia Bagni
Project role: Principal Investigator
Person months worked: 2 cal mos
Contribution to project: Design and supervise experiments and interpret data

Name: Esperanza Fernandez
Project role: Postdoctoral fellow
Person months worked: 9 cal mos
Contribution to project: Design, perform experiments and analyze data

Name: Giulia Cencelli
Project role: visiting graduate student
Person months worked: 3 cal mos
Contribution to project: perform experiments and analyze data

Name: Jonathan Royaert
Project role: Junior technican
Person months worked: 4 cal mos
Contribution to project: Breeding and genotyping of mice

Name: Karin Jonckers
Project role: Senior technican
Person months worked: 4 cal mos
Contribution to project: Breeding, genotyping of mice, brain slices for phosphorylation studies